Thyroid Hormone and Prolactin Profiles in Male and Female Turkeys Following Photostimulation

J. A. Proudman*,[1] and T. D. Siopes†

*Biotechnology and Germplasm Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705; and †Department of Poultry Science, College of Agricultural and Life Sciences, North Carolina State University, Raleigh, North Carolina 27695-7608

ABSTRACT The turkey hen, a photosensitive bird, will become photorefractory (PR) during the reproductive cycle and will cease laying despite a stimulatory day length. This response is thought to be "programmed" by hormonal events early in the reproductive cycle. The turkey tom, in contrast, produces semen for extended periods and has not been shown to exhibit PR. We compared hormone profiles following photostimulation of hens and toms to assess differences that might program one, but not the other, for PR. We photostimulated with 16 h light per day and measured plasma prolactin (PRL), thyroxine (T4), and triiodothyronine (T3) weekly for 12 wk, and again at 16 and 22 wk. Hens were fed ad libitum, and toms were moderately feed-restricted. Results showed increasing PRL levels following photostimulation in hens, with peak levels occurring at about the time of peak egg production, and declining thereafter. Toms maintained significantly lower concentrations of PRL ($P < 0.0001$) than hens after 2 wk of photostimulation. A highly significant sex by time interaction in plasma T3 levels was observed due to extreme fluctuations in males. Similar, often reciprocal, fluctuations in mean T4 concentrations also occurred in males. We recycled the toms and repeated blood collections under identical conditions, but with ad libitum feeding to determine if feed restriction may have produced these unusual results. This study revealed an initial significant decline in plasma T3 levels and an increase in T4 levels immediately following photostimulation, and then steady (T4) or slowly rising (T3) levels through 12 wk photostimulation. We conclude that PRL profiles of toms and hens differ markedly during the reproductive cycle, lending support to the suggestion that rising PRL may mediate the onset of PR. Further study is needed to determine if the low plasma T3 levels in males may be related to delayed PR. The extreme fluctuations in plasma T3 and T4 levels of toms receiving relatively mild feed restriction suggest a need for further study of the metabolic effects of feed restriction in turkeys.

(*Key words*: prolactin, thyroxine, triiodothyronine, turkey, photorefractoriness)

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INTRODUCTION

Reproduction in turkeys is controlled by photoperiod and is a balance between 2 physiological states, photosensitive and photorefractory (PR). The hen requires a period of short day lengths to establish photosensitivity (and terminate PR) and the ability to be photoinduced into egg laying, whereas the tom does not have a short day requirement and will mature and produce semen when given long days only (Polley et al., 1962; Singh, 1985). Furthermore, hens and toms are known to express the PR response differently; it is well established in hens but ill defined, if even present, in toms. In recent years, the most prominent hormones associated with PR have been prolactin and the thyroid hormones. The PR response is thought to be programmed by the presence of thyroxine (T4) during the early weeks following photostimulation (Nicholls et al., 1988; Wilson and Reinert, 1999; Dawson, 2001). The sex difference in PR response of turkeys is not common among birds, and provides an opportunity to differentiate hormonal influences on the expression of PR.

Thyroid hormones also have gonadostimulatory effects that promote photoinduced gonad development. Development times of ovaries and testes following photostimulation differ considerably in turkeys and this results in commencement of egg production 2 to 3 wk and semen production 4 to 6 wk postlighting. It is not known if sex differences in rate of gonadal development are associated with differences in circulating thyroid hormone levels. Interestingly, turkey hens require the presence of thyroid

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[1]To whom correspondence should be addressed: JohnP@anri.barc.usda.gov.

Abbreviation Key: PR = photorefractory/photorefractoriness; PRL = prolactin; T3 = triiodothyronine; T4 = thyroxine.

hormones for photoinduced ovarian development (Lien and Siopes, 1989a, whereas toms do not require thyroid hormones for testicular development (Lien and Siopes, 1991). The objective of our initial experiment was to compare the early, postlighting profiles in hens and toms of 3 hormones, T4, triiodothyronine (T3), and prolactin (PRL), to identify differences that might be associated with photoresponsiveness. A second experiment was conducted in an effort to investigate unexpected fluctuations in circulating T3 and T4 levels of males observed in the first experiment.

MATERIALS AND METHODS

Experiment 1

Males. Six BUTA Large White turkey toms, 60 wk of age, were recycled with a short photoperiod (8L:16D) for 10 wk. The toms were quantitatively feed restricted for the first 3 wk of short photoperiods to get a 20% decrease from starting BW. Thereafter, and until photostimulation, toms were fed one-half of the estimated ad libitum feed intake (based on a prior study in same environmental conditions) for maintenance. Body weights were taken to confirm that a stable weight level was achieved and maintained. Feed during the short day recycle period was an N.C. State University crumble ration calculated to contain 11.8% CP and 2,970 kcal of ME/kg. The feed used from photostimulation and beyond was a pelleted N.C. State University ration calculated to contain 15% CP and 2,970 kcal of ME/kg. Postlighting, toms were fed 0.45 kg of feed per day to maintain a slow, steady rate of gain in BW. The feed allotments were issued at 0800 to 0900 h on Monday, Wednesday, and Friday each week. Toms were photostimulated in the last week of July in a closed-confinement building with incandescent light only (44 to 55 lx, 16L:8D, with the light period starting at 0430 h). Floor pens had wood shavings and there was mechanical ventilation at ambient temperature. Heparinized blood samples were collected weekly by venipuncture in the afternoon (1300 to 1400 h).

Females. Nine Nicholas Large White breeder hens, 68 wk of age, were recycled with 8L:16D for 8 wk, then photostimulated with 16L:8D (light period starting at 0430 h) in the first week of September. Bird management and blood sampling were the same as for males except that postlighting all hens were fed an N.C. State University ration calculated to contain 16% protein and 2970 kcal of ME/kg of feed and the diet was ad libitum at all times.

Experiment 2

This experiment was done in direct response to the plasma thyroid hormone results of males in experiment 1. The purpose was to determine if the postlighting fluctuations in thyroid hormone levels of males in experiment 1 also occurred in full-fed males. All 6 toms of experiment 2 were recycled as in experiment 1 except that they were fed ad libitum at all times. The toms were 112 wk old and were photostimulated in the first week of August so that the time of year was the same as in experiment 1. All other conditions were as described in experiment 1.

Hormone Assays

Plasma levels of PRL, T4, and T3 were measured by RIA. Prolactin was measured using the homologous RIA of Proudman and Opel (1981). Thyroxine and T3 were measured using commercial kits[2] with modifications for turkey plasma (Siopes, 1997).

Statistics

The RIA data for weekly plasma hormone concentrations were analyzed for time (week), sex (male vs. female), and time by sex effects by the repeated-measures ANOVA using the GLM procedure of the SAS Institute (1990). When sex or time by sex effects were found, differences between means of the 2 groups within times were assessed using the GLM procedure.

RESULTS

In experiment 1, plasma PRL levels differed significantly with time following photostimulation (Figure 1; $P < 0.002$) and with sex ($P < 0.001$). There was a significant sex by time interaction ($P < 0.05$). Prolactin levels were low in males and did not vary as the reproductive season progressed. Hens showed a marked increase in PRL following photostimulation. Highest PRL levels were observed at about the time of peak egg production.

Neither T3 ($P < 0.17$) nor T4 levels ($P < 0.95$) differed significantly between toms and hens. Both hormones varied significantly with time ($P < 0.001$). A highly significant sex by time interaction was observed in plasma T3 levels ($P < 0.0001$). Both hormones varied much more in males than in females, and concentrations appeared to cycle on a 2- to 3-wk interval. Similarly, the T3/T4 ratio was quite constant in hens (Figure 2) but varied markedly over the reproductive cycle in toms ($P < 0.0001$).

In experiment 2, only males were studied, and neither T4 nor T3 showed the dramatic fluctuations observed in experiment 1 (Figure 3). Plasma T4 levels remained mainly within a range of 7 to 10 pg/mL throughout 12 wk of postlighting. This is comparable with the plasma range for T4 in experiment 1 (8 to 11 pg/mL). Plasma T3 declined by about 68% by the second week postlighting and remained low and stable (range: 0.3 to 0.6 pg/mL) for the remaining 10 wk of the test. This result contrasts with a plasma T3 range of about 1.0 to 2.0 pg/mL in experiment 1. The T3/T4 ratio was qualitatively similar to the T3 response.

[2]Diagnostic Products Corp., Los Angeles, CA.

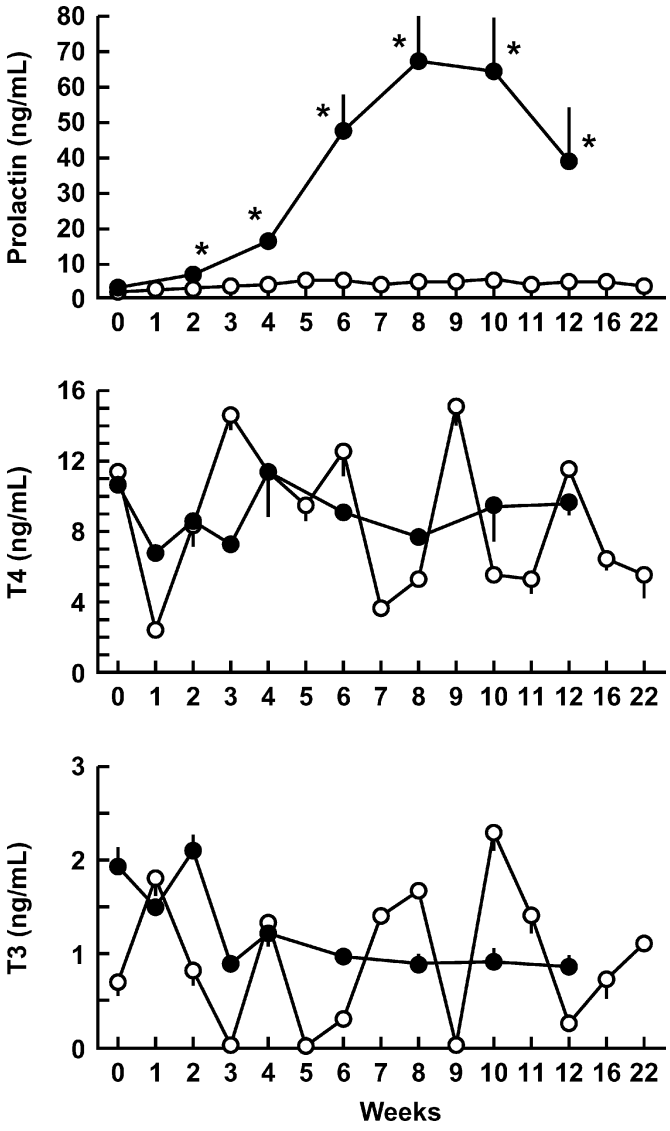

FIGURE 1. Plasma prolactin (PRL), thyroxine (T4), and triiodothyronine (T3) levels (mean ± SEM) of male (open circles; n = 6) and female turkeys (closed circles; n = 7 to 9) following photostimulation in experiment 1. Males were feed-restricted and hens were fed ad libitum. Asterisk indicates significant difference ($P < 0.05$) between sexes within time.

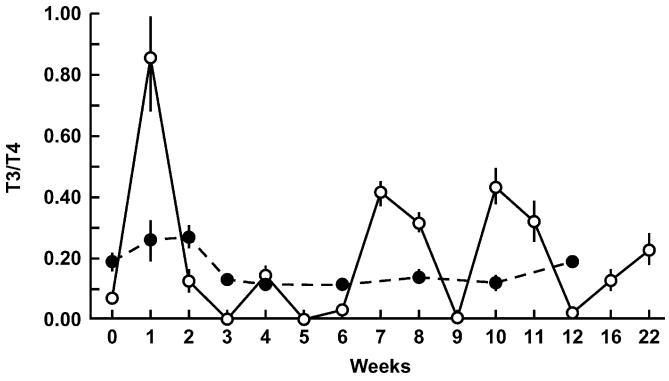

FIGURE 2. Ratio of plasma levels of triiodothyronine (T3) and thyroxine (T4) of male (open circles; n = 6) and female (closed circles; n = 7 to 9) turkeys following photostimulation (experiment 1). Males were feed-restricted and hens were fed ad libitum.

DISCUSSION

Our results show a highly significant sex difference in plasma concentrations of PRL following photostimulation. The PRL profile in hens was similar to that reported many times in turkeys; that is, PRL rises with photostimulation and reaches a zenith after peak egg production but before the onset of PR (e.g., Wong et al., 1991; Proudman, 1998). Earlier reports of PR in wild birds initially focused on PRL as a likely candidate for a PR-programming hormone precisely because elevated levels of PRL always preceded PR. Earlier studies in turkeys have shown that PRL increases in hens that become PR and those that remain photosensitive (albeit less so in PR hens), and that PRL levels subsequently decline more quickly in PR hens (Lien and Siopes, 1989b; Proudman, 1998).

In contrast, PRL has received little study in male turkeys, and our results show no increase in PRL following photostimulation. This is interesting because the presence of PR has never been established in toms. The situation is quite different in male starlings, a PR species, where PRL levels increase dramatically following photostimulation, reaching a peak at the start of gonadal regression (Dawson and Sharp, 1998). Interestingly, when Dawson and Goldsmith (1983) modified the onset of PR by changing photoperiod, they observed that males that became PR continued to show increased PRL levels immediately before the onset of PR, whereas males on a photoperiod that did not induce PR did not show an increase in PRL secretion. Taken together with the fact that PRL is antigonadotropic in birds (Camper and Burke, 1977; Opel and Proudman, 1980), these results suggest that PRL may be associated with the expression of PR in the turkey. The nature of this association may be that suggested by the model for neuroendocrine photoperiodic control of the breeding season proposed by Sharp and Blanche (2003). In this model, photostimulation independently activates the gonadotropin-releasing hormone-luteinizing hormone neuronal system to stimulate reproduction and, more slowly, the vasoactive intestinal peptide-PRL neuronal system, which ultimately increases circulating PRL to a level that depresses the gonadotropin-releasing hormone-luteinizing hormone system. The turkey tom would appear to lack activation of the vasoactive intestinal peptide-PRL system, thereby eliminating any photostimulation-induced increase in circulating PRL levels, delaying PR, and prolonging the breeding season compared with turkey hens. Studies in the male starling (Dawson and Sharp, 1998) showed that similar suppression of circulating PRL to very low levels (by immunization with vasoactive intestinal peptide, the hypothalamic releasing factor for PRL), markedly delayed the onset of PR, but that PR still occurred.

Studies in wild birds have shown that photorefractoriness requires both long day length and the presence of T4. Wilson and Reinert (1999) have shown that T4 acts through the brain to program the PR response. Although

T3 is thought to be the tissue-active form of thyroid hormone, studies by these authors and by Wilson (2001) concluded that T3 is relatively ineffective in programming PR compared with T4. However, Yoshimura et al. (2003) reported T3 in the mediobasal hypothalamus to mediate photoinduced gonadal development and suggested that high levels of T3 may down-regulate thyroid hormone receptors and induce photorefractoriness. Because turkey hens express PR but toms do not, and hens require thyroid hormones for photoinduced gonad development but toms do not, we reasoned that sex differences in circulating thyroid hormone levels may occur after PS and that any observed differences may provide evidence for the role of thyroid hormones in programming PR. Our first study revealed no significant sex difference in either T4 or T3 following PS, but a sex by time interaction in plasma levels of T3 was significant, whereas that of T4 approached significance. Plasma concentrations of T4 and T3 were quite stable in females, whereas levels of both hormones varied markedly from week to week in males. Peaks in plasma T3 levels in toms occurred somewhat regularly at approximately 3-wk intervals, whereas plasma levels approached zero at several other points. Changes in plasma T4 levels were often opposite those of T3, suggesting that changes in deiodinase enzyme activities could be involved. These reciprocal changes are perhaps best represented by the T3/T4 ratio. This ratio reflected consistent 5- to 10-fold higher levels of T4 than T3 in hens. However, in males the varying patterns of both hormones resulted in ratios that indicated a much higher proportion of T3 at some sampling points. These dramatic fluctuations in thyroid hormones of males were unexpected, and, because we know of no reports where plasma T3 is undetectable in normal physiology, we suspected that our routine feed restriction of the males might have caused the aberrant plasma thyroid hormone levels. Feed restriction in the chicken decreases plasma T3, increases plasma T4, and elevates hepatic type III deiodinase activity (Buyse et al., 2000, 2002). To confirm this suspicion, we repeated the first experiment using only males (the same males used in experiment 1), but provided feed ad libitum.

Experiment 2 revealed a significant decline in circulating T3 concentrations immediately following photostimulation in toms, followed by a gradual increase as the reproductive season progressed. Plasma thyroxine levels increased significantly following photostimulation and then remained steady. A direct comparison with the hens in experiment 1 is not possible, but the markedly lower levels of T3 in the male may warrant further study in relation to PR. The results of experiment 2 (Figure 3) are clearly free of the thyroid hormone fluctuations of experiment 1 and allow the interpretation that the fluctuations in the hormone levels in males in the first experiment were due to the limitation in daily feed intake. The severity of the effect on plasma thyroid hormone levels was somewhat surprising because the limitation of feed was minor (received 0.45 kg/tom per d) and BW was not only maintained, it increased slowly and consistently over

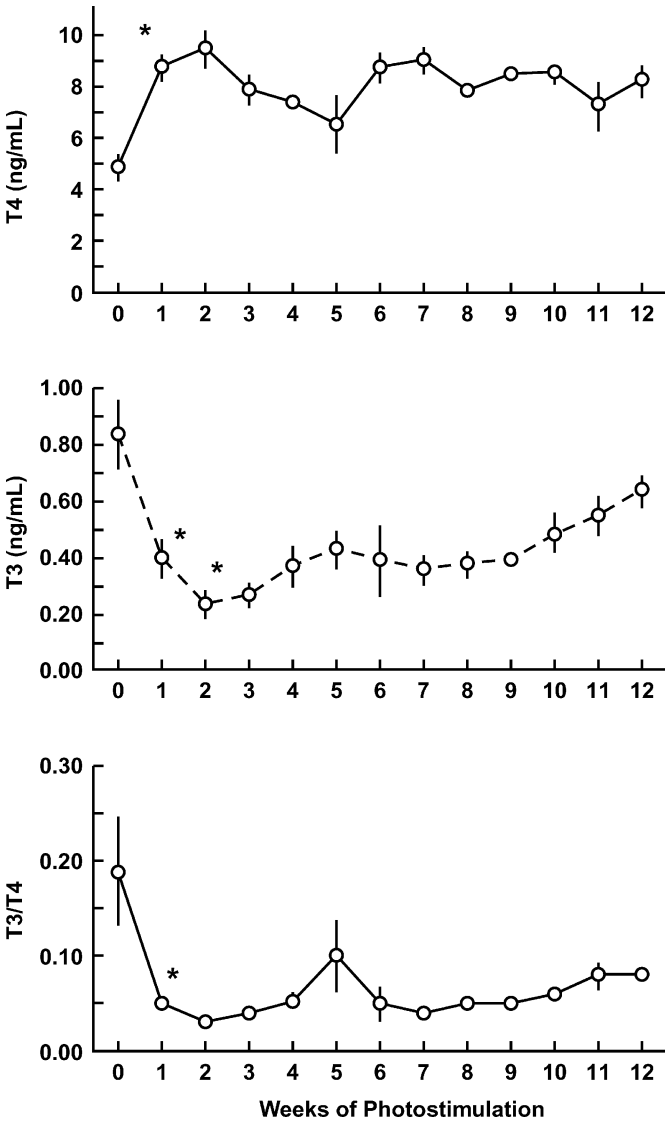

FIGURE 3. Plasma thyroxine (T4) and triiodothyronine (T3) concentration (mean ± SEM) and the T3/T4 ratio of ad libitum-fed male turkeys (n = 6; experiment 2). Asterisks denote values significantly different ($P < 0.05$) from preceding value.

the test period. Restricted feeding does result in meal feeding, which can alter thyroid hormone levels (Buyse et al., 2002), but the pre- and postprandial differences in T3 and T4 levels reported in meal-fed broiler chickens are mild compared with the wide fluctuations reported here. To our knowledge, the effect of feed restriction on thyroid hormone levels in turkeys has not been reported. Correlations of the peaks and valleys in T3 and T4 to specific procedural or physiological processes are outside the scope of this experiment. However, the results clearly indicated that thyroid hormones in male turkeys could be dramatically influenced by even minor alterations in feed intake. This is significant because of the importance of thyroid hormones as a growth and metabolic regulator (as well as a regulator of PR). It is common practice in turkey research and in commercial turkey production to apply some degree of feed limitation to males but not females. More research is needed to assess the effects of

feed limitations on thyroid hormones on males and how this affects their performance.

We conclude that postlighting profiles for PRL differ markedly between toms and hens, and that the low plasma PRL levels during reproduction in the tom (which did not exhibit PR) supports the suggestion of Sharp and Blanche (2003) that PRL may mediate the onset of PR. The role of thyroid hormones in programming PR remains unclear, but plasma T3 appeared to be consistently lower in full-fed males than in females.

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